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ANTIGENIC STRUCTURE OF GENUS *BRUCELLA*

Summary

Classification and nomenclature of *Brucella*. After the discoveries of *Brucella melitensis* (Bruce), *Brucella abortus* (Bang) and *Brucella suis* (Traum), the three microorganisms were treated separately. Evans (1918) proved on the basis of morphological, cultural and serological (agglutination) studies that all the three bacteria consituted a single genus or species, which was named *Brucella*. Different names are given in literature to types of *Brucella* isolated from goats, cattle and swine, with separate names being given even to the R-phase strains of those bacteria. Further serological (Wilson and Miles), biochemical and bacteriostatic (Huddleson) research has shown, that by means of this kind of research it is possible to differentiae between those three types of *Brucella*. Studies of types, carried out on a great number of *Brucella* strains, isolated in various countries (Lisbonne, Renoux, Zdrodowski), showed in those three types of *Brucella* a far-reaching variation of characteristics that had so far been considered fixed. A conception was put forward by Burnet, Renoux and others about the great dynamics of development and the great variability of *Brucella* bacteria, which are antigenically elastic. As a result of those studies, the modern nomenclature was introduced, the term: *Brucella brucei*, as well as the names of the variants: *melitensis*, *bovis*, *suis* and *intermedius*. Then, further descriptions of the variants appeared. Making a synthetic review of the above-mentioned studies and conceptions, we have worked out a tentative division of the *Brucella* variants with the idea in mind that it could undoubtedly become a beginning of a system of classification.

Tendencies are often encountered in literature to include bacilli *Pasteurella tularensis*, and even *Pasteurellae*, in the genus or even species of *Brucella*. Own morphological research, conducted in the Electron Microscope Laboratory of *Państwowy Zakład Higieny* (State Institute of Hy-

giene; collaborator: F e l t y n o w s k i), as well as antigenic studies, have shown that *Brucella brucei* on the one hand, and *Pasteurella tularensis*, *Pasteurella multocida* and *Pasteurella rodentium* on the other, belong to different species.

Views on the antigenic structure of the surface and interior of cells of *Brucella* bacilli. W i l s o n and M i l e s hold that in the surface layer of cells in phase „S" two antigenic substances (agglutinogens), namely A and M, are present. Generally speaking, both substances are found in all the varieties of *Brucella*, though not in equal quantities; in *Br. melitensis*, the proportion of A to M is 1 to 20; in *Br. bovis* and *suis*, the proportion is reversed: A : M = 20 : 1.

Thus, quantitative proportions are the decisive factor in the typical differences between strains. While the two above mentioned authors, as well as many others, tend to consider these quantitative and qualitative proportions as stable, almost unchanging, own observations and studies lead us to the opinion that the proportions can be experimentally changed, and that they do change in *Brucella's* natural habitat, both quantitatively and qualitatively.

On the basis of an analysis of receptors of thousands of strains, isolated in various parts of the world, R e n o u x and M a h o f f e y in 1950 put forward another conception of the dislocation of antigenic substances in *Brucella* varieties.

In our opinion, the problem demands further studies, which would determine whether those conceptions are right.

Results of own research into the antigenic structure of *Brucella* varieties found in Poland. The problem is of great importance not only theoretically, but also from the practical point of view. It is a question of paramount importance in epidemiology and epizootiology whether, along with the bovis variety, also strains of the *melitensis* and *suis* varieties and intermediate strains are to be found on Poland's territory. To find an answer to this question, the receptors of about 150 strains of own collection, isolated in Poland, as well as standard strains from abroad, were subjected to an analysis, in which monovalent sera of *antimelitensis* and *antibovis - suis* were used; also biochemical (CO_2 requirement, H_2S production, catalase and urease activity, growth on cow, goat and woman's milk) and bacteriostatic (aniline dyes) methods were applied. Much care was devoted to a standardization of those methods, which was of fundamental importance, if errors were to be avoided. Monovalent sera were obtained in our laboratory for the needs of the whole country. The bacteriostatic method was modified. As a result of those studies, an important thing was found: among Polish strains, one typical and one atypical variety of *melitensis*, 5 typical and 4 atypical varieties of *suis*, as well

as 20 atypical intermediate strains of the *bovis* variety, were detected. Two typical strains of the suis variety were isolated by J. Brill from swine afflicted with *brucellosis* and aborting on a mass scale on one of the state farms.

Attempts at transforming described variety characteristic *in vitro*. Three standard strains were used in research: the *melitensis*, *bovis* and *suis* varieties, which are characterized by typical serological, biochemical and bacteriostatic properties. Out of dense suspensions of those strains, which had been disintegrated by means of supersonic vibrations, dead substrates were obtained. On those substrates, heterologous strains were passaged over a period of many weeks. As a result of the passages (which might be called metabolic-antigenic crossing), we have come to interesting findings: standards strains of *melitensis*, *bovis* and *suis* have undergone biochemical and bacteriostatic change in the direction of intermediate, atypical varieties. The behaviour of these changed strains in receptor analysis was interesting; strains that had so far, in accordance with Wilson and Miles' thesis, undergone agglutination only under the influence of a monovalent serum of *antimelitensis* or *antibovis-suis*, now, as a result of many passages on antigenically heterological substrates, acquired the properties of intermediat new strains and agglutinated in almost the same titer with *antimelitensis* and *antibovis-suis* monovalent sera.

Immunochemical studies. Dubrowskaja (Biochemical Department, Institute of Microbiology and Epidemiology, Soviet Academy of Medical Sciences in Moscow), who carried out research into the antigenic substances of *Brucella* bacilli varieties using the Boivin method, has tried to present a conception of the antigenic structure of *Brucella* bacilli in the form of a scheme based on the quantitative biochemical composition of the *bovis*, *suis* and *melitensis* varieties.

By means of the method of paper chromatography, we have tried to ascertain differences in the range of composition of aminoacids and polysaccharides in the *melitensis*, *bovis* and *suis* varieties, and in the non-virulent variety of S 19 and PD; for the sake of comparison, the same method was applied to the bacilli of *Pasteurella tualerensis*, *Pasteurella multocida* and *Pasteurella rodentium*. The results of the analysis testify to certain differences in the composition of some polysaccharides, in particular of galactose, glucosamine, mannose and xylose; it is possible that those differences exert an influence on both the virulence and the serological properties of the varieties of the above - mentioned bacilli.

In all the strains of own collection, catalase activity was studied by the Huddleson method in own modification; urease activity was studied by the methods of Rustigian and Nessler. The results

of research into catalase activity (manganometric method) generally conformed to Huddleson's results and should be used in determining the virulence of strains, on the condition, of course, that this be only an element of examination; the bacteriological, immunobiological and histopathological picture, produced by the particular strain in guinea pigs in the various periods of the disease, is the basis of examination and of virulence. Further immunochemical research was concerned with protein--polysaccharide complexes and the protein and polysaccharide fractions of *Brucella*, and the latters' essential role in the complement-fixation test, the hemagglutination and precipitation tests and skin-allergic reactions.

The polysaccharide fractions are the best and fundamental antigen in the complement-fixation test. This antigen is probably placed deep inside the cell. The polysaccharide fraction is special specific, and, at the same time, common to the varieties of *Brucella*. Own numerous experiments, which were based on the methods of Burnet, Huddleson, Castaneda, Ottosen and Plum and which aimed at obtaining haptens (protein-free complexes) as specific allergens, causing Burnet's positive skin-allergic reaction in brucellose infected men and animals, have shown (the tests on men and animals) that this kind of cell--derived allergens are special specific, but common and identical in the individual varieties of *Brucella* bacilli.

The same result was achieved in studies of own non-antigenic allergens M and NM, which came from own strains. Comparative allergometric studies, carried out on men and animals, point to the diagnostical superiority of Brucellin PD, obtained through the desintegration of cells by means of supersonic vibrations.

Variability in the shapes of *Brucella* colonies and antigenic properties. All the strains from own collection and imported strains of *Brucella* were studied morphologically (in some cases, in an electron microscope); main attention was paid to the characteristics of *Brucella* colonies and antigenic characteristics. The colonies were first examined according to all kinds of tests; later, only three methods were applied (Henry's in own modification, Braun's and Burnet's).

The studies confirmed the results of Henry, Braun, Huddleson, Zdrodowski, Renoux and others; also among the populations of Polish strain colonies there is usually a preponderance of phase S colonies, which are antigenically complete. The dissociation of strains is a universal phenomenon and it tends in the direction of the opposite phase, namely phase R, which lacks many features of antigenic specifity. Between those two phases there exist a wide range of intermediate phases — I. In own collection of Polish strains eleven types were in this way

described; the paper is documented in this respect with photographs and tables.

Most of those types have been described in literature. In addition to them, we have found new ones which have not been described; these are going to be shown on photographs.